A fluorescence *in situ* hybridization method using a peptide nucleic acid probe for the detection of *Salmonella* spp. in biofilms

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Abstract

A novel peptide nucleic acid (PNA) probe for the detection of *Salmonella* spp. has been developed. The probe was synthesized and the Alexa Fluor dye 594 was attached to the N-terminus in order to allow detection by fluorescence *in situ* hybridization (FISH). Specificity and sensitivity probe matching theoretical estimates were both of 100%. The PNA FISH method was optimized, and laboratory testing on representative strains from the *Salmonella* genus subspecies and several related bacterial species, confirmed the predicted theoretical values of specificity ans sensitivity. Afterwards, the method was successfully adapted to cell detection in suspensions and biofilms. Counterstaining with 4′,6-diamidino-2-phenylindole (DAPI) allowed *Salmonella* spp. discrimination from heterotrophic consortia of bacteria. However, the direct detection in biofilms presented some limitations for particular types of adhesion materials. These limitations were mainly related with the autofluorescence of the support material at the same wavelength emission as the probe. Nevertheless, this limitation has been overcome by disrupting the biofilm (sonication step) and performing the hybridization on glass slides or in suspension. We hence conclude that PNA FISH represents a reliable tool for biofilm study, allowing specific and direct detection for most support materials, and hence provides spatial organization information for specific groups of microorganisms within mixed/natural biofilms for substrata without a strong autofluorescence signal.